GENETIC CONTROL OF INSECT POPULATIONS: I. CAGE STUDIES OF CHROMOSOME REPLACEMENT BY COMPOUND AUTOSOMES IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

A genetic method for insect control was evaluated using the test organism, *Drosophila melanogaster*. The technique involved the displacement under a system of continuous reproduction, of standard strains by those carrying compound autosomes. The eradication of the replacements could subsequently be achieved through the use of temperature-sensitive lethal mutations.——While certain compound autosome strains failed to displace standards in population cages, even at the initial release ratio of 25:1, others were highly successful. Indeed, for some strains when the ratio of compounds to standards was as low as 9:1, the population rapidly went to fixation in favor of the compound line.——Hatchability was found to be an insufficient index of fitness to estimate the initial ratios of compounds to standards that would guarantee fixation of the former. Differences in other fitness components, such as development time, were detected that could seriously modify displacement, especially with continuous overlapping generations. The importance of examining the fitness of various compound lines and selecting the most competitive in cages, prior to field tests, cannot be overemphasized.

TO overcome many of the drawbacks of conventional control methods, increasing attention is being focussed upon the development of genetic techniques for the suppression of insect pests. One specific genetic method of insect population control currently under examination by several laboratories involves the replacement of normal chromosomes by chromosome rearrangements carrying either lethal factors for subsequent eradication or mutants which render the replacements innocuous (CURTIS 1968; WHITTEN 1971; FOSTER *et al.* 1972; CHILDRESS 1972; FITZ-EARLE 1972).

Hybrids formed between strains bearing certain chromosome rearrangements (e.g., translocations and compound autosomes) and standard lines (where standard is defined as carrying non-rearranged chromosomes) show reduced viability. In a mixed population in which there is no mating isolation of rearrangements from standards, there is intrastrain fertility but a degree of interstrain sterility. In a competitive situation, one can adjust the proportions of the two

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strains according to their relative fitness such that the mixed population will theoretically be in a state of unstable genetic equilibrium (LI 1955). If the proportion of the rearrangements exceeds the equilibrium value, the population is expected to be driven to fixation in favor of the chromosomally-rearranged strain. Under the assumption that generations are discrete, replacement of the standard strain should be completed within very few generations, depending upon the relative fitnesses of the rearrangements and standards (WHITTEN 1971). However, if generations are continuous or overlapping, the model requires considerably higher proportions of rearrangements for equilibrium, and a longer time to achieve fixation (FITZ-EARLE 1973). During population replacement, the fertility will be reduced due to the genetic load imposed. However, this does not necessarily imply that the replaced population's size will be diminished, due to the possibility of buffering by density-dependent factors.

One candidate for application in population replacement regimes is a special type of translocation termed the compound autosome which was first described by RASMUSSEN (1960). Aspects of the construction, stability and meiotic behaviour of compounds have been investigated in the fruitfly, *Drosophila melanogaster* (for a complete review see HOLM 1973), and studies of these arrangements are being considered for other insect species. Whereas standard chromosomes have their homologous arms attached to different centromeres, compound autosomes have their homologous arms attached to the same centromere. Progeny arising from crosses between compound and standard strains have zero fitness (i.e., have complete interstrain sterility), since they carry duplications and deficiencies for the arms which render them lethal during the zygotic stage of development.

At meiosis in *D. melanogaster*, 95% of the gametes produced by females carry left or right disjunctional compound arms, whereas the remaining 5% carry diplo- or nullo-nondisjunctional gametes. By contrast, in males, the four classes of gametes are produced in equal frequency (25%) BALDWIN and CHOVNICK 1967; HOLM, DELAND and CHOVNICK 1967). The expected zygotic viability of a compound line will be approximately 25%; that is, on the basis of hatchability, compounds will have a fitness of one-fourth that of standards. In theory, if the equilibrium ratio of compounds to standards is 4:1, an increase of the compound frequency will result in the replacement of the standards. Thus, compound autosomes will be used as vehicles through which other genetic factors may be introduced into pest populations (FOSTER *et al.* 1972; FITZ-EARLE 1972). One class of factors which may be driven into populations is temperature-sensitive (ts) lethal mutations. These have been studied extensively in *D. melanogaster* (SUZUKI 1970), and have also been detected in the housefly *Musca domestica* (MCDONALD and OVERLAND 1972) and in the predaceous wasp, *Habrobracon serinopae* (SMITH 1971).

Temperature-sensitive lethal genes exist which permit Drosophila to survive at permissive temperatures (e.g., 22°C) but which lead to death at restrictive temperatures (e.g. heat-sensitive, 29°C, cold-sensitive, 17°C). Temperature-sensitive mutants have been detected throughout the Drosophila genome; some have

relatively short temperature-sensitive periods (tsp), others will die following a temperature shock at any time during development. The tsp does not necessarily coincide with the time at which death occurs (lethal phase) but may be separated from the expression of lethality by several days.

If a native standard strain is replaced by a compound line carrying a ts mutant at the permissive temperature, then the newly-introduced strain will be eliminated when exposed to the low or high temperatures likely to occur during the seasonal fluctuations that follow. The recent finding that one-third of all autosomal ts lethals on chromosome 3 recovered in *D. melanogaster* are both heat- and cold-sensitive (TASAKA and SUZUKI 1973) is of interest here. A strain carrying such a lethal mutation, when introduced into the wild, would be subjected to temperature stresses both in the winter and summer months.

Since *D. melanogaster* is the only organism in which the requisite genetic tools are available at this time, it is being used in a program of laboratory and field experiments. These studies will provide the theoretical and experimental bases for which similar genetic constructs may be used with other insects. This initial paper prescribes the laboratory replacement of standard strains of *D. melanogaster* by compound lines in population cages in which there were overlapping generations. Subsequent publications will examine the results of replacement and elimination experiments with laboratory and native lines both in the laboratory and in the field.

MATERIALS AND METHODS

Stocks: The laboratory strains of *Drosophila melanogaster* used in the experiments are listed in Table 1. All compound autosomes are reversed metacentrics and therefore the symbol *RM* has been omitted. The acronym VH2, for example, is the code designating the location, discoverer and number of the compounded arm, i.e., V—Vancouver, S—Storrs, P—Pasadena, H—Holm, K—Kiceniuk. For a complete description of the markers used, see LINDSLEY and GRELL (1968).

Population cages and growth conditions: The cages used in the investigation were 18″ plexiglass cubes. The top had a 7″ × 7″ air vent covered with screening and the front had a port enclosed by a sleeve that permitted access to the flies and the food trays inside. Food trays contained 50 mls standard cornmeal-agar medium. Each of the seven trays per cage was introduced sequentially every 3 days; the oldest tray, therefore, remained in the cage no longer than 21 days. A dish of water was included to maintain high humidity and the temperature was held at 24.5 ± 0.5°C.

Mark-recapture experiments using sterile X/O males marked with *y* (yellow body color), showed that the cages at carrying capacity were capable of maintaining in the order of 20,000 flies.

TABLE 1

The compound and standard chromosome arrangements of Drosophila melanogaster *used in the experiments*

Compound 3	Standard 3	Compound 2	Standard 2
C(3L)VH2,ri;C(3R)SH19,+	+ (Oregon-R)	*C(2L)SH3,+;C(2R)VK2,bw*	+ (Oregon-R)
C(3L)SH2,+;C(3R)SH19,+	*ri*	*C(2L)VH1,lt;C(2R)VK2,bw*	
C(3L)VH2,ri;C(3R)VK1,e^s		*C(2L)VH2,lt;C(2R)P,px*	
C(3L)VH3,st;C(3R)VK1,e^s			

TABLE 2

The genotypes and ratios of standard and compound chromosomes initially introduced in cage tests

Compound	Initial no. pairs	Standard	Initial no. pairs	Initial ratio*	Initial frequency†
C(3L)VH2,ri;C(3R)SH19,+	60	+	10	6:1	.859
C(3L)VH2,ri;C(3R)SH19,+	70	+	10	7:1	.875
C(3L)VH2,ri;C(3R)SH19,+	90	+	10	9:1	.900
C(3L)VH2,ri;C(3R)SH19,+	120	+	10	12:1	.923
C(3L)VH2,ri;C(3R)SH19,+	150	+	10	15:1	.938
C(3L)VH2,ri;C(3R)SH19,+	250	+	10	25:1	.961
C(3L)VH2,ri;C(3R)SH19,+	250	+	10	25:1	.961‡
C(3L)SH2,+;C(3R)SH19,+	250	*ri*	10	25:1	.961
C(3L)VH2,ri;C(3R)SH19,+	Equilibrium§	+	50	—	—
C(3L)VH2,ri;C(3R)VK1,e^s	250	+	10	25:1	.961
C(3L)VH2,st;C(3R)VK1,e^s	250	+	10	25:1	.961
C(3L)VH2,st;C(3R)VK1,e^s	150	+	10	15:1	.938
C(2L)SH3,+;C(2R)VK2,bw	250	+	10	25:1	.961
C(2L)SH3,+;C(2R)VK2,bw	150	+	10	15:1	.938
C(2L)SH3,+;C(2R)VK2,bw	90	+	10	9:1	.900
C(2L)VH1,lt;C(2R)VK2,bw	250	+	10	25:1	.961
C(2L)VH1,lt;C(2R)VK2,bw	150	+	10	15:1	.938
C(2L)VH1,lt;C(2R)VK2,bw	90	+	10	9:1	.900
C(2L)VH2,lt;C(2R)P,px	250	+	10	25:1	.961
C(2L)VH2,lt;C(2R)P,px	150	+	10	15:1	.938
C(2L)VH2,lt;C(2R)P,px	90	+	10	9:1	.900

* Compounds:Standards
† Frequency of compounds
‡ Two day advantage for compounds
§ Ongoing cage of compounds

Fitness component studies: For certain strains, 30 males and females were aged for one day, then mated in single pairs in glass shell vials. The females were permitted to lay eggs for 24 hours, after which they were transferred to fresh food and the total number of eggs was recorded. Non-hatched eggs were counted after a further 36 hours. All offspring were scored and the time and date of emergence were noted. Egg production, hatch and adult eclosion were measured over the reproductive lifespan of the females, though only pooled hatchability data for days 5–10 and 16–21 are reported here. Mean numbers of offspring per female per day were calculated and graphed over the lifetime, and egg-adult survival values were computed for the two 6-day periods. Mean development time was estimated for progeny derived from eggs laid over the life of the females.

Experimental design: Unmated compound and standard, males and females, were aged for 3–4 days and then released into cages in the initial ratios given in Table 2.

Sampling scheme: Beginning about 14 days after establishment of flies in a given cage, and subsequently every 3 days until fixation, a random sample of approximately 300 flies was extracted with an aspirator. The flies were anesthetized, scored according to presence or absence of a marker and then returned to the cage, in order to leave unaltered the population structure of the cage. Fixation was considered to have been reached when three consecutive samples (taken over a 9-day period) yielded only standard or compound individuals. The ratio of compound flies to total flies in each sample was calculated and the frequency of compounds was plotted against time in days.

RESULTS

Fitness component studies: The hatchability at peak egg production (days 5–10) for *C(3L)VH2,ri:C(3R)SH19,+* was 24.9%, a value which agreed favorably with those found in other studies for *C(3L)SH3,+;C(3L)SH20,+* and *C(3L)SH2,+;C(3R)SH21,+* (Table 3).

Larval and pupal death in the line *C(3L)VH2,ri;C(3R)SH19,+* from eggs laid during the interval 5–10 days led to a survival value of approximately 18%. From the same strain, during a later period in the females' lifetime (days 16–21), the hatchability was reduced to 19.1% whereas the survival remained at 18%. By contrast the standard strain had a hatch of 95% during the earlier period and 93% during the later one. The survival was approximately 80% for days 5–10 and 86% for days 16–21. In terms of both hatchability and survival, the fitness of standards was in the order of 4–5 times that of compounds for chromosome 3, a finding which is consistent with the view that meiosis in compound 3 females is regular, while in males it is random.

Hatchability measurements over days 5–9 for various compound 2 intercrosses and outcrosses (Table 3) were significantly higher than 25%. The mean hatch value was 27.7%, indicating that there may be differences between compound 2 and compound 3 lines in meiotic behavior.

The mean number of offspring per female is plotted over time in Figure 1 for the compound strain *C(3L)VH2,ri;C(3R)SH19,+* and the standard strain. The standard began producing offspring from eggs laid earlier than the compound; the offspring production rapidly reached a peak of 20 per female per day and declined to zero by day 40. The *C(3L)VH2,ri;C(3R)SH19,+* line, however, began

TABLE 3

Fitness components of selected compound and standard strains

Strain	Period*	Percent hatch	Percent egg-adult survival	Generation time
C(3L)VH2,ri;C(3R)SH19,+	5–10	24.9	18.1	12 days
C(3L)VH2,ri;C(3R)SH19,+	16–21	19.1	18.4	12 days
+	5–10	95.1	79.7	11 days
+	16–21	93.2	86.2	11 days
C(3L)SH3,+;C(3R)SH20,+	5–9	23.2	21.7	— †
C(3L)SH2,+;C(3R)SH21,+	5–9	25.6	—	— †
C(2L)SH1,+;C(2R)SH1,+	5–9	27.8	—	— ‡
C(2L)SH3,+;C(2R)SH3,+	5–9	27.6	—	— ‡
C(2L)SH3,+;C(2R)SH3,+ ♀ × *C(2L)P,b;C(2R)P,px* ♂	5–9	27.3	—	— ‡
C(2L)P,b;C(2R)P,px ♀ × *C(2L)SH3,+;C(2R)SH3,+* ♂	5–9	29.9	—	— ‡
C(2L)P,b;C(2R)P,px	5–9	26.1	—	— ‡

* Days post eclosion
† Data of HOLM and CHOVNICK 1973a
‡ Data of HOLM and CHOVNICK 1973b

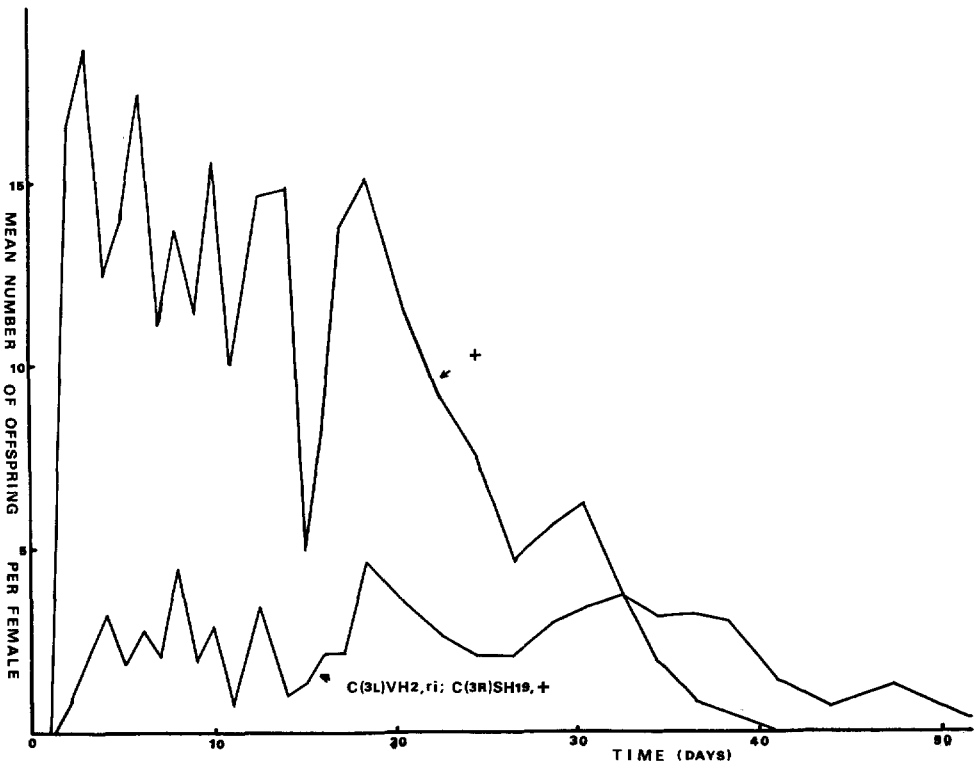

FIGURE 1.—Mean number of offspring per female from compound line *C(3L)VH2,ri; C(3R)SH19,+* and a standard strain + over time in days.

producing later and never had an output exceeding 5–6 offspring per female per day, but sustained this rate until shortly before cessation at day 50. It is interesting to note that the offspring ratio of standards:compounds is very high during the first few days of offspring production (e.g., 20.9:1, 9.4:1, etc.) but the ratio declines and even reaches values < 1 in the later periods of production (e.g., 1.8:1, 1.0:1, 0.6:1, etc.).

Another observation consistent with the offspring results was that progeny from the standard line take one day less to develop than individuals from the *C(3L)VH2,ri;C(3R)SH19,+* strain (Table 3). This difference in development time was true of all eggs laid by females throughout their productive lifetimes (50 days for compounds; 40 days for standards). Although a consistent result was obtained with a second compound 3 line homozygous for *ri* (FITZ-EARLE, unpublished) it is possible that such differences will not be detected for other compound lines.

Compound 3's: It was anticipated that the compound strains, when in competition with standards in initial ratios greater than 4:1, would be driven to fixation, and that the replacement of the standards would take relatively few days. In fact, preliminary cage experiments with *C(3L)VH2,ri;C(3R)SH19,+* and +

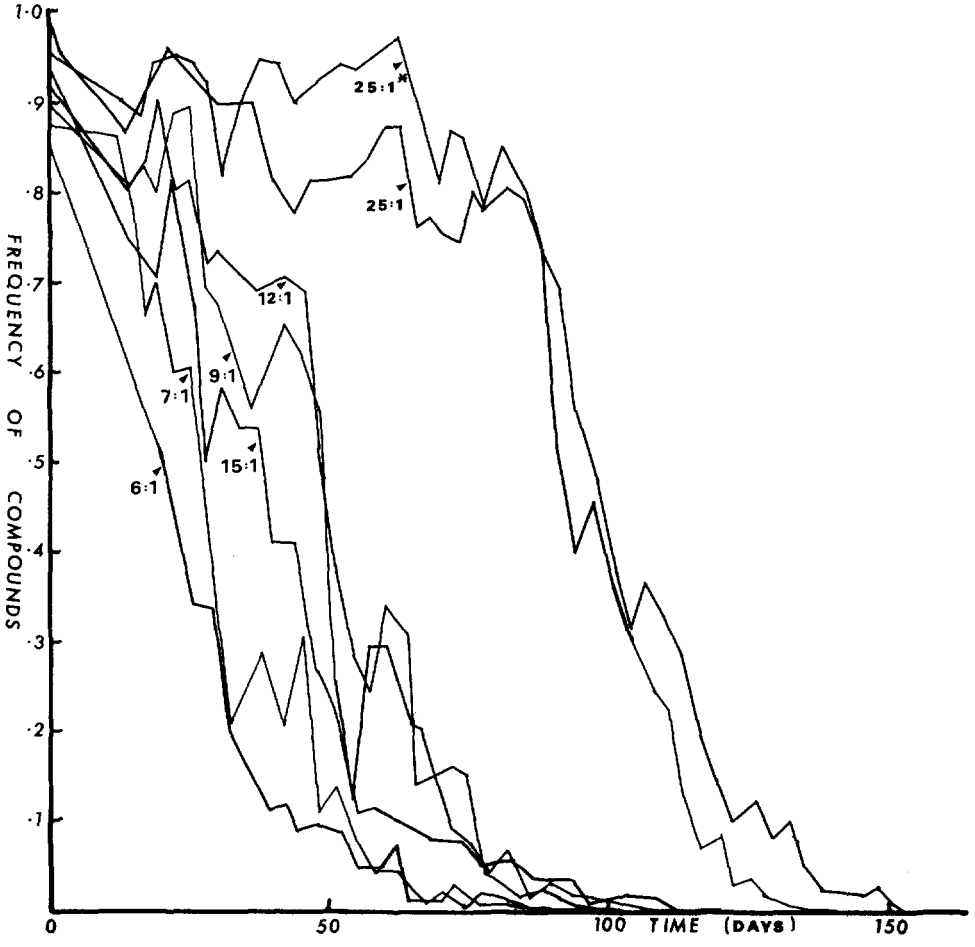

FIGURE 2.—Cage competitions between *C(3L)VH2,ri;C(3R)SH19,*+ and + in the initial ratios 6:1, 7:1, 9:1, 12:1, 15:1, 25:1 and a 25:1 in which compounds were given a two-day advantage (indicated by *).

with ratios 6:1, 7:1, 9:1, 12:1, 15:1 and 25:1 failed to show the expected displacement of the standard by the compound line (Figure 2). Additionally, a 25:1 in which the compounds were released two days before the standards also was unsuccessful. It was immediately clear that sheer weight of numbers was not a sufficient factor to fix a population of this strain of compounds.

Several reasons for the initial failures may be cited. The fitness component study had revealed that the mean generation time was extended in the compound strain by approximately one day compared to the standard. Consider, for example, that in the first generation following release, newly-emerging offspring of standards could mate with each other and with old standard parents, which would contribute to the standard pool, even prior to the availability of sexually mature compound progeny. Naturally, those standards which mated to old com-

pound parents would yield no offspring. Because of the developmental delay noted for the compound line, the earliest compound females to emerge might encounter a mating pool in which the sexually mature males from the standard rather than the compound line were predominant. Thus it follows that such compound females, over their initial phase of reproduction, would fail to contribute to the population replacement.

It was noted above that compound females seemed to produce offspring for approximately ten days longer than standards. The additional production might counteract the depressing effect of the more rapid development of the standards, but these two factors will be out of place. Indeed, the curves of compound freuency relative to standard, plotted over time, exhibited a cyclic behavior in compound frequency, indicating that the standard could take precedence but that the compounds could recover their numbers a few days later (Figure 2). In these experiments, however, the recovery was not sufficient to compensate for the effect of early emergence of standards.

One possible solution to the dilemma would be to select compound lines for shorter generation time (i.e., rapid development). Cage experiments to test a number of such selected compounds are currently in progress. A line which develops rapidly in culture, however, may not necessarily behave in a similar manner under competition in a cage.

It should also be noted that artificial compensation for developmental time difference (the 25:1 cage, in which the compounds were given a two-day advantage initially [Figure 2]), failed to overcome the difficulty for the strain, *C(3L)VH2, ri;C(3R)SH19,+*. This result would seem to lend support to the view that the standard line also develops more rapidly than the compound line under cage conditions and thus overcomes the initial temporal disadvantage. Furthermore, under the crowded conditions of the cage at carrying capacity, the difference in development time between the two strains may even show greater divergence. This could explain the more rapid displacement of the compound line following day 80 (Figure 2).

The failure of the compound line to successfully replace the standard may be a function of the marker *ri*. To test this possibility, a cage was established using *C(3L)SH2,+;C(3R)SH19,+* and a standard strain, homozygous for *ri*, with an initial ratio of 25:1. In this case, successful elimination of the standards was achieved in 40 days (Figure 3), thus supporting the contention that the marker may have imposed a selective disadvantage upon the compound line. Additional evidence that the *ri*-marked compound arm was partly responsible for failure of the replacements was to be found in two further experiments. One cage was established with *C(3L)VH2,ri;C(3R)VK1,e*^{s} and + in the ratio of 25:1, two others contained *C(3L)VH3,st;C(3R)VK1,e*^{s} and + in the ratios of 25:1 and 15:1. As in the initial experiments, the compounds bearing the *ri* marker failed to displace the standards, whereas the compounds bearing *st* instead of *ri* successfully replaced the standards in both ratios examined.

It should be emphasized that each compound arm, as a function of its formation, may possess properties that are unique. Thus, the success of the compound

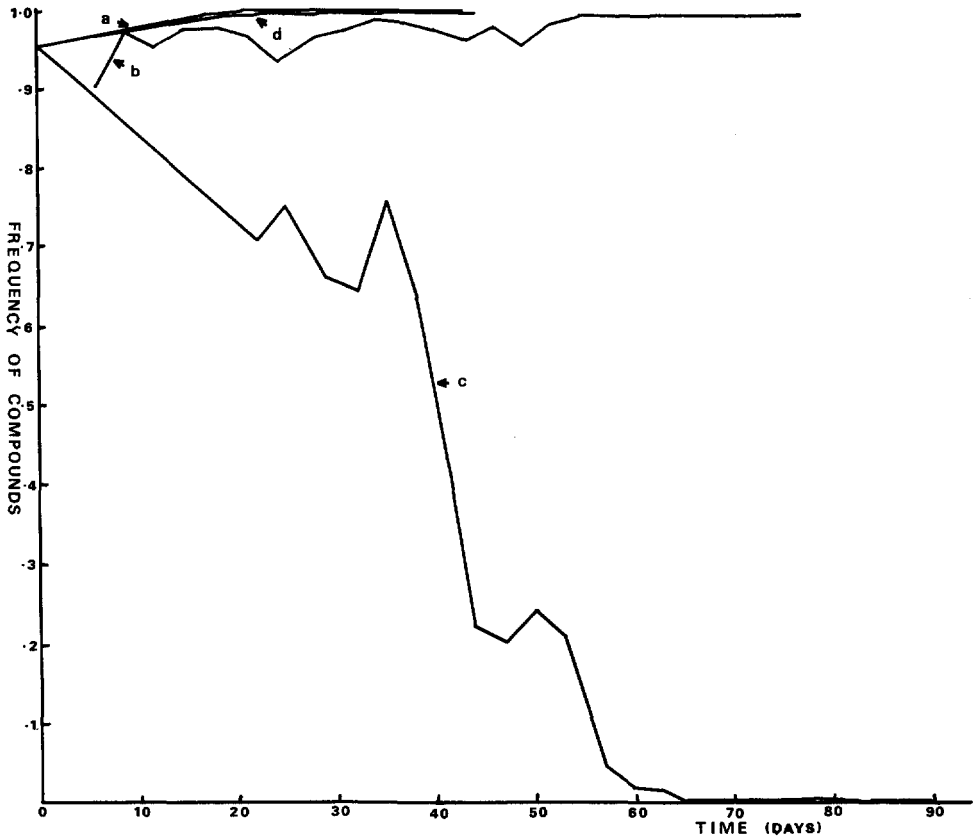

FIGURE 3.—Cage competitions of (a) *C(3L)SH2,+;C(3R)SH19,+ vs. ri* with a 25:1 initial ratio; (b) *C(3L)VH2,ri;C(3R)SH19,+* established, then seeded with 50 pairs +; (c) *C(3L)VH2, ri;C(3R)VK1,e*^{s} *vs.* + with a 25:1 initial ratio; and (d) *C(3L)VH3,st;C(3R)VK1,e*^{s} *vs.* + with a 25:1 initial ratio.

carrying the *st* gene in the above experiments may be quite unrelated to the gene itself, but may be a function of the compound arm, its relationship to the complementary compound or, indeed, to the entire genome.

Since no successful displacements were obtained using the *C(3L)VH2,ri; C(3R)SH19,+* line for the range of ratios 6:1 to 25:1, the extreme possibility that the strain would always be eliminated in spite of their numbers was tested as follows. A cage of compounds only was permitted to increase to carrying capacity of the cage; the population was then seeded with 50 pairs of unmated standards. After 80 days, the standards were completely eliminated and the dynamic population of compounds alone was restored (Figure 3). Thus, a population of *C(3L)VH2,ri;C(3R)SH19,+* can be buffered against 'invasions' of standards but the ratio of compounds to standards required may be prohibitively high.

Compound 2's: Compound autosomes generated from second-chromosome

material in *D. melanogaster* are known to differ from compound 3's both in their fitness and in their meiotic behavior (HOLM and CHOVNICK 1973a,b). Whereas in compound 3's hatch is $\leqslant 25\%$, all compound 2's have hatch values higher than 25% (Table 3). Cultures of compound 2's in the laboratory display qualitative superiority in vigor to compound 3's. The higher hatch and increased fitness of compound 2's relative to compound 3's may be attributable to a unique feature of the former. Some compound 2's are known to carry duplications of small proximal segments of the one arm on the other arm and *vice versa* (KOWALISHYN 1971; HOLM *et al.* 1973). For example, the strain *C(2L)VH2,lt; C(2R)P,px* has been shown to carry $Dp(lt^+)$ on its right arm and $Dp(rl^+)$ on its left arm (HOLM *et al.* 1973; GIBSON 1973). These duplications may contain pairing sites homologous to those on the alternate arm. The homology may lead, at meiosis, to a degree of pairing in males (known to be completely random in compound 3's), resulting in higher hatch and increased fitness.

Three strains of compound 2's, *C(2L)SH3,+;C(2R)VK2,bw*, *C(2L)VH1,lt; C(2R)VK2,bw* and *C(2L)VH2,lt;C(2R)P,px* have each been competed against + in the initial ratios 25:1, 15:1 and 9:1. All compounds have successfully and rapidly replaced the standards in the cages. The initial ratios were seemingly so far in excess of the minimum replacement frequencies that there were no meaningful differences between the rates of replacement (Figure 4). Elimination took an average of 50 days for the *lt;bw* strain, about 52 days for the +;*bw* line and approximately 48 days for the *lt;px* compound.

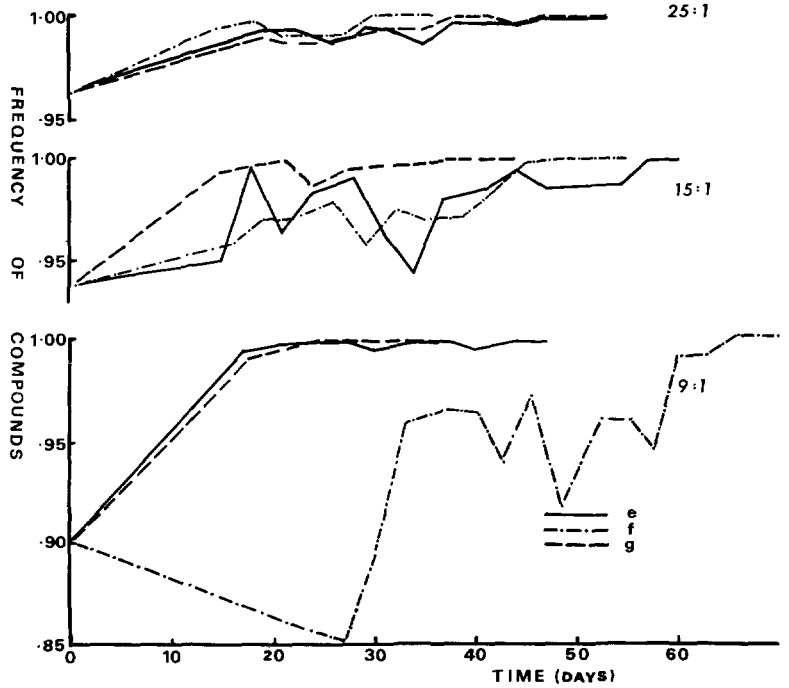

FIGURE 4.—Cage competition of (e) *C(2L)SH3,+;C(2R)VK2,bw;* (f) *C(2L)VH1,lt; C(2R)VK2,bw;* (g) *C(2L)VH2,lt;C(2R)P,px vs.* + and in the initial ratios of 25:1, 15:1, 9:1.

DISCUSSION

The comprehensive examination of competitive ability in cages and fitness components of various compound 2 and compound 3 strains of *D. melanogaster* revealed several interesting features. Under a system of continuous reproduction, compound autosome lines can replace standard lines in population cages. Indeed, for certain strains, cages, in which the initial ratios of compounds to standards were as low as 9:1, speedily went to fixation of the compounds. Hatchability alone is not a sufficient index of fitness on which to base estimates of initial compound: standard ratios.

Differences which were detected between compounds and standards, in such fitness components as development time, reproductive lifespan, rate of offspring production and total production, could seriously modify replacement over continuous generations.

Development time differences between compounds and standards (found for certain strains to be in the order of one day) would give an advantage in terms of displacement to the earlier emerging line. Additionally, it is well established, and has been supported in these studies, that the reproductive lifespan of flies is at least three times as long as the development time. In native populations, therefore, at any given point in time, individuals from several age groups will be present. Thus, the cage conditions in these experiments are more realistically simulating wild populations of fruitflies, in which there is considerable generation overlap, than those conditions encountered in experiments dealing with discrete generations. Furthermore, it is not possible to extrapolate from a system involving discrete generations to a system of continuous reproduction, since generation length has no meaning in cages of the latter class, and the effects of exponentially-increasing population size through to carrying capacity would not be manifested in the former.

The potential for imposing a genetic load upon a natural population and replacing the population with a translocation bearing a desirable genotype was originally postulated by CURTIS (1968). He argued that since translocation heterozygotes are semi-sterile but translocation homozygotes are fully fertile, in a mixed population of translocations and standards, the strain with the higher frequency should be favored by selection. The system has recently been examined in Drosophila by ROBINSON and CURTIS (1973). Two cages, each containing translocations and standards in the initial ratio of 9:1, were tested over time for fertility and translocation frequency. Although there was a noticeable degree of reduction in population fertility, the translocation was eliminated from both cages. The failure of the replacement was attributed to the reduced viability of the translocation homozygote in the competitive environment. Differences in other fitness components such as development time and reproductive lifespan could also have seriously influenced the success of the translocation experiments, in an analogous way to the compound case. Due to the failure of the translocation competitions, the cage studies with compound autosomes described in this paper remain the only successful demonstration of displacement of standards by chromosome rearrangements in populations of Drosophila with overlapping generations.

FOSTER *et al.* (1972), using bottled cultures, and CHILDRESS (1972), using cages, have described the displacement of standards by compounds over discrete generations. In a series of experiments with a compound 2 strain carrying the wing marker *dp*, FOSTER *et al.* (1972) found that initial ratios in the order of 9:1 compounds to standards or higher would successfully drive the compounds to fixation in relatively few generations. The unstable equilibrium frequency was not 0.8 (equivalent to a 4:1 ratio), as expected, but approximately 0.9; the discrepancy was accounted for on the basis of the effect of the *dp* marker or intrinsic effects of the chromosome rearrangement. A similar observation was made by CHILDRESS (1972) who used a *dp;px* compound line.

Additionally, a strain carrying unmarked compound 2's, but having the *X* chromosome marked with a body color mutant, *y*, was competed in cages against unmarked standards in the initial ratios of 3:1 and 10:1, each experiment being replicated once (CHILDRESS 1972). The apparent success of 3:1 initial ratios of compounds to standards in these experiments leads to the speculation that meiosis in the males was not random (i.e., equal proportions of disjunctional 2L, 2R and nondisjunctional 2L and 2R, O gametes) but that possibly the disjunctional gametes were produced in higher frequencies due to increased pairing. The majority of compound 2's tested have hatch values in the range of 26%–29% (HOLM and CHOVNICK 1973b). CHILDRESS's (1972) compound strain would have required a hatchability of 33% or greater for replacement of standards using the ratio of 3:1. Only two records of hatches in excess of 30% have been described; CLARK and SOBELS (1973) reported hatchability of 31% for a compound 2 strain, while EVANS (1970) described a line with 41% hatch.

CHILDRESS (1972) chose a strain bearing a marker on the *X* chromosome rather than on the compound arm, for cage replacements of a standard line. In addition to the primary nondisjunction of the *X* chromosomes occurring with a frequency of 0.1%, GIBSON (1973) has shown that nondisjunction of certain structurally-homozygous second chromosomes is of the same order of magnitude. Indeed, the frequency of spontaneous nondisjunction of a $+/ap^{wow}$ strain was 0.113%. Although nondisjunction would not have affected CHILDRESS's (1972) experiments, if the population sizes had been larger, there is a possibility that the *X* chromosome marked with *y* would be transmitted to the standard. If a compound strain, therefore, is to be an effective vector for elimination of natural populations, it is clear that the controlling factor (e.g., temperature sensitivity) must be linked to the compound autosome. Otherwise, there is a risk that a significant proportion of the compound strain will lose this controlling factor.

There is no way of assessing whether there is assortative mating within a cage. However, the fact that all cages in the studies reported in this paper went to fixation of compounds or standards (as defined in MATERIALS AND METHODS), supports the argument that if assortative mating was taking place, it was insufficient to alter the results. Three of CHILDRESS's (1972) cages gave frequencies of compounds of 0.923, 0.976 and 0.944 at the termination of the experiments. The possibility exists that these frequencies may have been maintained subsequently

due to assortative mating. Male Drosophila carrying the y gene apparently do not have the behavioral repertoire that induces unmarked females to accept them as mates (BASTOCK 1956; BARKER 1962). Thus, a compound line which bears the y marker on its X chromosome may exhibit a degree of mating isolation from its unmarked standard competitor in a cage.

Another reason why it is essential to take cage experiments to fixation, according to our definition, is well illustrated in Figure 2 by the cage involving *C(3L)VH2,ri;C(3R)SH19*,+ and + in the initial ratio of 25:1. After a considerable period of time, the compound line in this experiment reached a frequency of 0.981. Shortly thereafter, however, a rapid reversal took place, leading to fixation of the standard strain. If the 0.981 value had been interpreted as fixation, the subsequent elimination of compounds would have been completely overlooked.

The experiments in the present paper illustrate only one of two likely situations that may be encountered in the field. Establishing cages in which fixed initial ratios of compounds and standards are set up (i.e., founding parents), essentially simulates the situation in which an insect undergoes reproductive arrest or diapause during the winter, and compounds are introduced just before or during the early stages of exponential growth. Alternatively, some insects may be encountered in a constant reproductive state, their abundance only fluctuating with environmental changes. In such ongoing populations, recruitment from preadult stages in the target strains could dramatically alter the efficiency of the release regime. The population dynamics of caged strains and the evaluation of various replacement strategies applied at different stages of increase, as well as at carrying capacity, are currently under investigation.

The laboratory investigations described above are a necessary, but not a sufficient, prelude to the application of the genetic control technique under field conditions. There are a number of non-genetic factors which must also be considered before a realistic field program can be initiated. It is essential to examine such parameters as the population dynamics of native strains, the ecology of an infested area, the migratory and dispersive activity of the insects, and the behavioral patterns of the pests. To this end, a continuing series of field studies, that will complement the laboratory experiments, is being conducted to evaluate those factors which may modify the replacement of Drosophila in the wild. These field investigations are indicating the most likely strategies for the control of test populations in the wild. Field replacement trials to evaluate such release regimes, using native strains bearing compound autosomes and temperature-sensitive lethal mutants, are being developed.

The laboratory and field studies described above are designed to develop a general method that may be adapted in the future to programs for the genetic regulation of pest insects of both hygienic and economic significance. The possibility of utilizing population replacement through compound autosomes, followed by suppression or elimination by temperature-sensitive lethal mutations, is being considered for the various root maggot flies, *Hylemya sp.*, by this labora-

tory, as well as for the sheep blowfly, *Lucilia cuprina* in Australia (FOSTER 1972) and the filariasis mosquito, *Culex p. fatigans*, in India (R. PAL, personal communication).

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